SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 28, No. 11, pp. 2255–2262, 2009

Printed in the USA
0730-7268/09 $12.00 + .00

ARSENIC BINDING TO IRON(II) MINERALS PRODUCED BY AN IRON(III)-REDUCING *AEROMONAS* STRAIN ISOLATED FROM PADDY SOIL

XIN-JUN WANG,† XUE-PING CHEN,† ANDREAS KAPPLER,‡ GUO-XIN SUN,† and YONG-GUAN ZHU*†§
†State Key Lab of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 10085, China
‡Geomicrobiology, Center for Applied Geosciences, University of Tübingen, Tübingen 72076, Germany
§Key Lab of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China

(*Received* 20 *February* 2009; *Accepted* 22 *May* 2009)

Abstract—An iron-reducing bacterial strain was isolated from a paddy soil and identified as a member of the *Aeromonas* group by 16S rRNA gene sequence analysis. When the cells were growing with dissolved Fe(III) as the electron acceptor in the presence of As(V), Fe(II) minerals (siderite and vivianite) were formed and dissolved. As was removed efficiently from solution. When the cells were growing with the Fe(III) hydroxide mineral (ferrihydrite) as the electron acceptor in the presence of As(V), ferrihydrite was reduced and dissolved As(V) concentrations decreased sharply. The present study results demonstrated first that members of the *Aeromonas* group can reduce Fe(III) in paddy soils and second that iron reduction does not necessarily lead to arsenic mobilization. However, As immobilization can occur in environments that contain significant concentrations of counterions such as bicarbonate and phosphate.

Keywords—*Aeromonas* Arsenic Dissimilatory iron-reducing bacteria Iron minerals Paddy soil

INTRODUCTION

Iron (Fe), one of the most abundant metals on Earth, comprises nearly 5.6% of the Earth's crust. Iron(II) can function as an electron donor, and Fe(III) can function as a terminal electron acceptor in anoxic environments. Thus, the redox change between Fe(II) and Fe(III) is an important biogeochemical process in anoxic soils and sediments. However, earlier studies suggested that iron reduction was a result of abiotic reactions, at least until dissimilatory iron reduction was more clearly described [1]. Dissimilatory iron reduction is considered to be an important process in which dissimilatory iron-reducing bacteria can gain energy for growth by coupling the oxidation of organic compounds or hydrogen to the reduction of ferric iron oxides. This biochemical reaction has a profound influence on the global biogeochemical cycling of elements, including carbon (C), sulfur (S), nitrogen (N), and phosphorus (P) in addition to heavy metals and arsenic (As) [2–5].

Dissimilatory iron-reducing bacteria have been described in various environments, including freshwater [6], marine sediments [7], natural wetlands [8], and contaminated aquifers [9,10]. In paddy soils, however, little is known about the microorganisms responsible for anaerobic iron respiration, especially in calcareous paddy soils.

Paddy soil is intermediate between upland systems and true aquatic systems whose alternation of flooding and drainage causes periodically occurring redox reactions. After flooding, oxygen is consumed by aerobic bacteria and chemical oxidation reactions, and most regions of the soil become anoxic. Alternative electron acceptors exist in the anoxic zone, with Fe(III) concentration often exceeding that of others, such as NO_3^- and SO_4^{2-}, and therefore serving as an important electron acceptor. Weiss et al. [11] showed that the percentage of poorly crystalline Fe(III) was correlated significantly with the percentage of iron-reducing bacteria, which accounted for 12% of total bacteria cells in the rhizosphere of wetland plants. Moreover, high levels of dissolved Fe(III) were observed in paddy soils [12]. Therefore, dissolved and solid Fe(III) are available in the rhizosphere for iron-reducing bacteria.

Arsenic is a toxic metalloid widely distributed in various environments, most notably in groundwater in southeast Asia [13]. Intensive use of As-contaminated groundwater for irrigation can cause increasing levels of As in paddy soils and As accumulation in rice grains [14,15]. Recent studies have shown that As can be sequestered in iron plaques on the root surfaces of rice plants [16–18]. The structure of the iron plaques has been characterized as amorphous or crystalline Fe(III) (oxy)hydroxides, which may be preferred substrates for iron-reducing bacteria. It has been demonstrated that dissimilatory iron reduction plays a key role in As release into waters from shallow reducing aquifers and paddy soils by reducing and dissolving Fe(III) minerals [19–21]. Moreover, it has been reported that iron-reducing bacteria colonized the root surface [11]. However, the effect of microbial iron reduction occurring in the rhizosphere on As mobility has not been completely elucidated and understood. In particular, the fate of As under conditions at which Fe(II) minerals are formed during Fe(III) reduction is unclear.

Therefore, the goal of the present study was not only to isolate and characterize an iron-reducing microorganism from paddy soil but also to investigate interactions among iron-reducing bacteria, dissolved and solid Fe(III), and arsenate.

MATERIALS AND METHODS

Soil samples

Soil samples were taken from the subsurface of a rice field (Tongzhou District, Beijing, North China Plain, 39°49.411′N,

* To whom correspondence may be addressed (ygzhu@rcees.ac.cn).
Published on the Web 7/2/2009.

116°36.912′E). When the soil was collected, it was placed into polyvinylchloride bottles, submerged with water imitating field conditions, and then transported back to the laboratory.

Culturing techniques and media

Standard anaerobic culturing techniques were used throughout the experiments [22]. In the present study, an anoxic carbonate-buffered (30 mmol/L) freshwater medium was used that contained as the following components (per liter of distilled water): NaCl, 1 g; $MgCl_2 \cdot 6H_2O$, 0.4 g; $CaCl_2 \cdot 2H_2O$, 0.1 g; NH_4Cl, 0.25 g; KH_2PO_4, 0.2 g; KCl, 0.5 g. After being autoclaved and cooled under an atmosphere of N_2–CO_2 (90:10, v/v), 30 ml of bicarbonate solution (1 M, autoclaved under CO_2), trace elements mixture (1 ml), vitamin mixture (1 ml), and a selenite–tungstate solution (1 ml) were added [22]. To limit the growth of sulfate-reducing bacteria, sulfate and sulfide were substituted by 100 μmol/L cysteine as a sulfur source. A Widdel flask was used for batch preparation of anoxic medium and dispensation into culture bottles. All incubations were carried out in serum bottles capped with thick butyl rubber stoppers and aluminum caps. The same mineral medium was utilized to test anaerobic growth.

Enrichment and isolation

For enrichment of iron-reducing bacteria, anoxic mineral medium with 10 mmol/L sodium acetate was used as the electron donor, and 30 mmol/L ferric citrate was used as the electron acceptor. All enrichment cultures were inoculated with 1% (v/v) soil slurry prepared in medium or in sterile distilled water. For isolation of the pure strain, dilution series were prepared. After several days, the highest dilution tube was consecutively diluted and plated on an agar plate containing the carbonate-buffered mineral medium with 10 mmol/L sodium acetate and 30 mmol/L ferric citrate. Iron-reducing bacteria were purified by streaking on ferric-iron-containing agar plates that were incubated in anoxic jars. All preparations were performed inside an anoxic glove box. Iron(III) reduction activity was recognized easily, because the growth resulted in clearing zones around the colonies. Ferric citrate was used as a basis for selection of the strain for further investigation.

16S rRNA gene sequencing and phylogenetic analysis

Bacterial DNA was isolated from cell pellets of the individual strain grown in the medium. DNA isolation was performed using standard procedures [23]. The DNA was used as a template for amplification of nearly the entire 16S rRNA gene (~1,500 base pairs) with the bacteria-specific primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and R1492 (5′-TACGGYTACCTTGTTACGACTT-3′). The polymerase chain reaction program was as follows: 95°C for 5 min and then 32 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, followed by a 10 min extension time at 72°C. The amplification products were confirmed by agarose gel electrophoresis and sequenced (Sangon Biotechnology). The 16S rRNA gene sequence of the strain has been deposited in GenBank under the sequence accession number EU696781. Phylogenetic analyses were performed with Molecular Evolutionary Genetics Analysis (MEGA4.0) software (Arizona State University). Trees were reconstructed using the neighbor-joining method. Robustness of derived groupings was tested by the bootstrap method using 1,000 replications.

Growth determination

Growth of the isolated strain on ferric citrate (3 mmol/L) and acetate (5 mmol/L) was monitored by measuring the increase in Fe(II) and cell protein (see below). For growth experiments, six bottles (36 ml) containing mineral medium were incubated equally with 4 ml of a culture grown on ferric citrate and incubated in the dark at 30°C. Three bottles were used for measuring Fe(II); the others were used for determining the content of cell protein. There was a control with no addition of ferric citrate.

Iron reduction experiments

Cells were grown in bicarbonate-buffered mineral medium containing acetate (5 mmol/L) as the electron donor and ferric citrate (3 mmol/L) or ferrihydrite (7.5 mmol/L) as the electron acceptor. Ferrihydrite was prepared using $FeCl_3$ and NaOH [24] and identified by X-ray diffraction (data not shown). Sodium arsenate (150 μmol/L) and anthraquinone-2,6-disulfonate (AQDS, 100 μmol/L) were added from anoxic stock solutions (4.5 mmol/L; 3 mmol/L). Anthraquinone-2,6-disulfonate is a functional analog for quinine moieties in humic acids. It acts as an electron shuttle to overcome the need for microbes to be in direct contact with Fe(III) oxides, potentially accelerating the reduction of poorly soluble Fe(III) minerals [24]. A 10% inoculum was used throughout, and cultures were incubated in the dark at 30°C.

Transmission electron microscopy and scanning electron microscopy of the precipitates

Samples (2 ml) of the cultures were fixed with glutaraldehyde (2.5%) for 4 h at 4°C [25]. Cell suspensions were centrifuged (3,381 *g*) for 5 min. The supernatant was discarded, and the pellet was washed three times in phosphate buffer (0.1 M, pH 7.4) and progressively dehydrated with solutions containing 30, 50, 70, 85, 95, and 100% ethanol in water. The washed suspension (5 μl) was transferred via a pipette onto a copper net, and a transmission electron microscope (H-7500, Hitachi) was used to image the cells. The same suspension was mounted onto a copper sample holder and coated with gold for scanning electron microscopy (S-3000N, Hitachi). An energy-dispersive spectroscopy system (EDAX Genesis) was used to determine the major elements present in the biominerals.

X-ray diffraction of the precipitates

X-ray diffraction was used to identify the precipitates that had been formed in the iron reduction experiments. The settled mineral residue was removed from the reduction experiments and dried under anoxic conditions. The dried solid was smeared on a glass slide and ground into fine slurry with the addition of a few drops of amyl acetate. The sample was then analyzed using a D/max 2500 diffractometer with Cu Kα radiation (Rigaku). Slides were kept under an anoxic atmosphere until further analysis.

Analytical techniques

Gram test was performed by Gram's method. Light microscopy images were taken using an AxioVison microscope (Carl Zeiss,) and an oil immersion objective lens [25]. Protein was quantified using the Bio-Rad Protein Assay with bovine serum albumin as a standard [26]. Iron minerals were removed by HCl (6 M) before cell protein determination. Iron(II) was

monitored over time by the colorimetric reagent 1,10-phenanthroline [27]. Approximately 1 ml of sample was collected from growing cultures and acidified for 15 min with 0.5 M HCl prior to iron and As analysis. Arsenic was measured from 0.5 ml aliquots after filtrating samples with a 0.22-μm filter by atomic fluorescence spectrometry (AFS-2202E, Beijing Haiguang Co.) [17]. Samples (1 ml) for quantification of dissolved and total As were removed from bottles in an anoxic cabinet. One aliquot (0.5 ml) for determination of As(III) and As(V) in solution was passed through a 0.22-μm filter prior to analysis by high-performance liquid chromatography inductively coupled plasma mass spectrometry (HPLC-ICP-MS) [28]. The other aliquot (0.5 ml) for total As(III) and As(V) was acidified with 0.5 M HCl for 15 min to dissolve the iron minerals, then analyzed by HPLC-ICP-MS.

Data analysis

All data were subjected to ANOVA using SPSS® 13.0 for Windows.

RESULTS

Enrichment and isolation

Iron(III) reduction occurred in the enrichment culture containing dissolved Fe(III) citrate. This was indicated by a change in the medium color from brownish gray to green due to the production of Fe(II). After complete Fe(III) reduction, a whitish precipitate was observed, probably consisting of ferrous carbonate (siderite) and ferrous phosphate (vivianite).

After several transfers, an aliquot of the highest dilution culture was transferred onto an agar plate. Uniform colonies were red, domed, entire, smooth, and wet, and cell morphology was obtained. Cells of single colonies were Gram-negative, single, straight rods. The cell size ranged from 2.3 to 2.5 μm in length by 0.3 to 0.5 μm in width (Fig. 1). The pure culture was isolated and preserved in liquid mineral medium with acetate and ferric citrate. The iron reducer was designated as strain BJ.

16S rRNA gene sequencing and phylogenetic analysis

The sequence of the 16S rRNA gene was compared with those of reference organisms obtained from GenBank. The result revealed that the isolated strain BJ is a member of the *Aeromonas* subphylum of Gram-negative bacteria (Fig. 2). The 16S rRNA gene of *Aeromonas hydrophila* had the highest similarity (99%) with that of strain BJ.

Growth determination

Significant cell growth and Fe(III) reduction were noted over 7 d (Fig. 3A). Protein content in BJ cultures increased significantly by the addition of Fe(III) citrate as an electron acceptor to the growth medium. There was a significant correlation between protein content and Fe(II) concentration (Fig. 3B). In addition, little or no growth was observed in control cultures in the absence of ferric citrate throughout the period (Fig. 3A). The maximum amounts of Fe(II) and cell protein were obtained on the fourth day. Approximately 60% of Fe(III) was reduced during this time period.

Interactions between As with biogenic Fe(II)

The experiments focused on actively growing cultures inoculated into medium containing 3 mmol/L ferric citrate or 7.5 mmol/L ferrihydrite as the electron acceptor and supplemented with 150 μmol/L As(V). In the presence of 150 μmol/L As(V), strain BJ reduced 1.59 ± 0.23 mmol/L Fe(III) within 7 d. After addition of AQDS, 2.66 ± 0.08 mmol/L Fe(II) was produced by strain BJ within 7 d (Fig. 4A). Microbial Fe(III) reduction was increased significantly by AQDS ($p < 0.05$) (Fig. 4A). Arsenic concentration in solution did not change within the first 2 d; however, it sharply decreased from 150 μmol/L to 16.16 ± 6.75 μmol/L on the third day (Fig. 4B). At the end of the experiments (8 d), approximately 90% of the dissolved As(V) was removed from the medium with the precipitate in the presence and absence of AQDS. There were no differences in dissolved As concentrations between the two treatments with and without AQDS. Arsenic(V) reduction was found in the experiment on the second day (Fig. 5). Arsenic(III) concentration in solution increased, whereas total As(III) concentration did not change after the third day (Fig. 5).

Compared with ferric citrate, ferrihydrite was reduced more slowly by strain BJ ($p < 0.05$) (ANOVA results not shown). The rate and the extent of bacterial Fe(III) reduction were significantly increased by AQDS ($p < 0.05$) (Fig. 6A). No

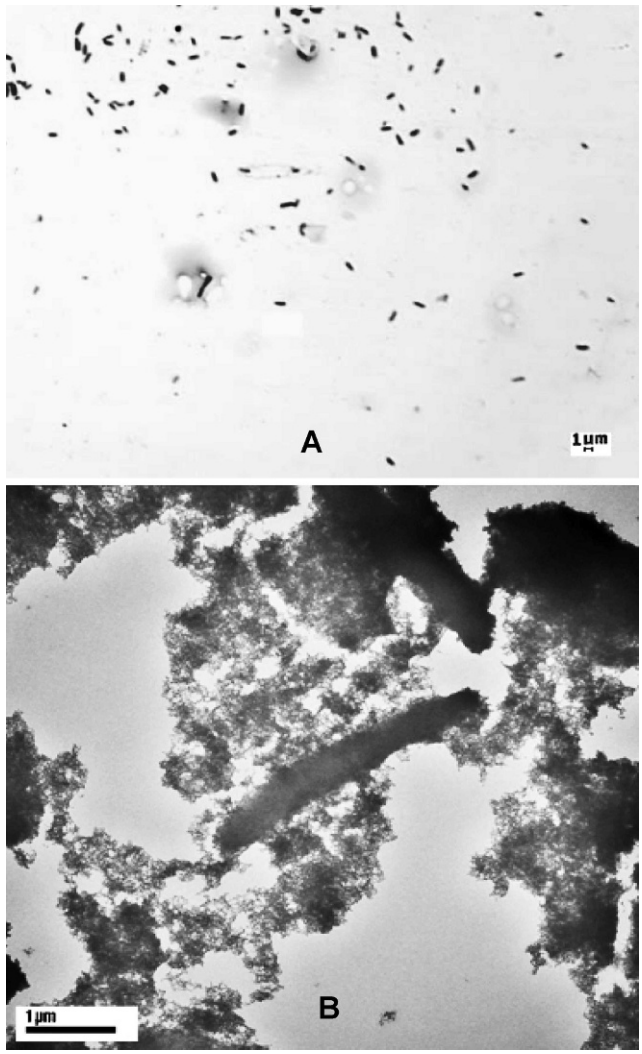

Fig. 1. (**A**) Light microscopy image of strain BJ cells at the beginning of reduction of Fe(III) citrate. The isolated *Aermononas* strain was designated as strain BJ. Scale bar, 1 μm. (**B**) Transmission electron microscopy images of BJ cells reducing ferric citrate. Sample was taken at the end of the reduction. Scale bar, 1 μm.

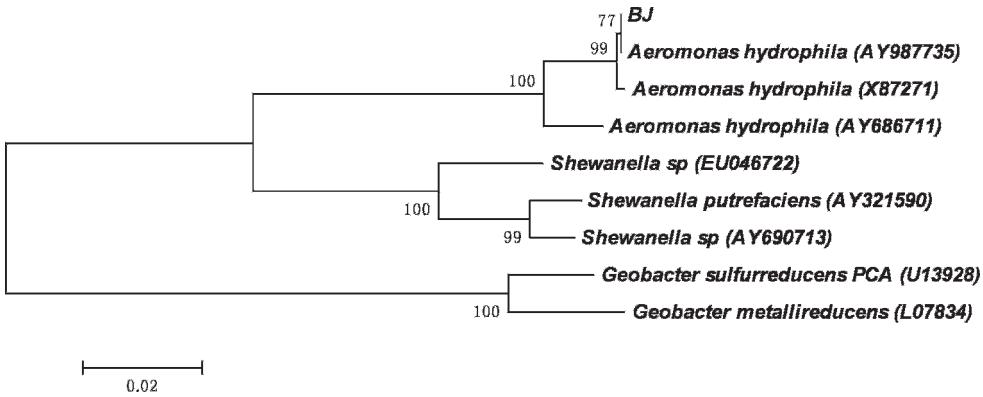

Fig. 2. Phylogenetic tree inferred from 16S rDNA sequences. The length of the bar represents one evolutionary distance unit.

reduction of Fe(III) was found in the absence of acetate. Arsenic concentration decreased from 150 to 25 μmol/L directly after the addition of ferrihydrite, suggesting significant sorption of As to the ferrihydrite (Fig. 6B), whereas 3 μmol/L and not more than 1 μmol/L As were detected in abiotic and biotic treatments at the end of incubation.

Identification of the mineral precipitates

Transmission electron microscopy showed that strain BJ was surrounded by extracellular mineral precipitates (Fig. 1B) at the end of reduction of ferric citrate. Energy-dispersive spectroscopy analysis of the precipitates (Fig. 7) indicated that

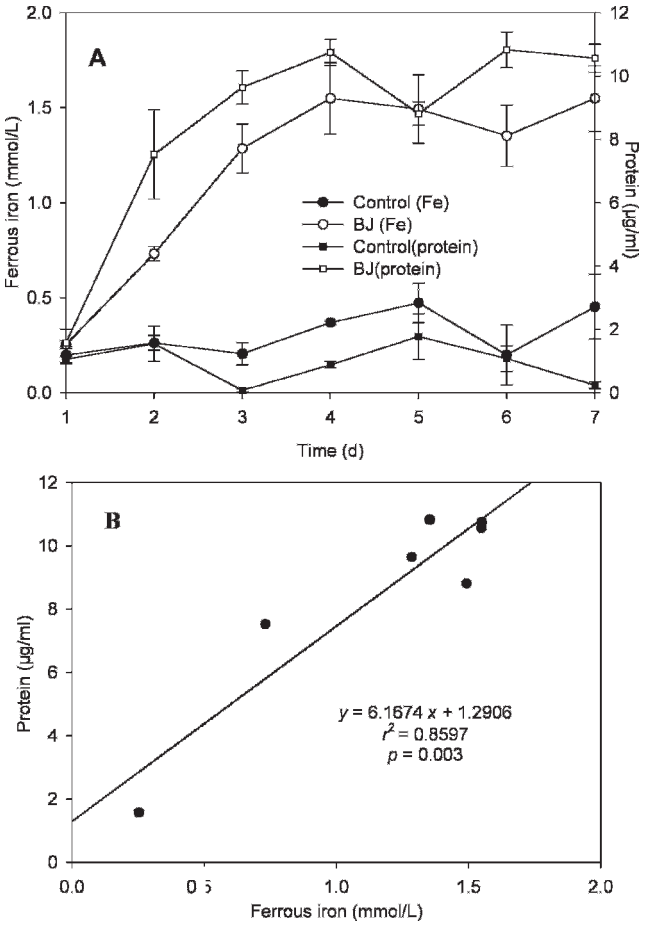

Fig. 3. (**A**) Growth of strain BJ in mineral medium with 3 mmol/L ferric citrate and 5 mmol/L acetate as the electron donor. The isolated *Aermononas* strain was designated as strain BJ. Growth was monitored by increase of Fe(II) and cell protein. Data are means of triplicates; error bars represent standard error. Iron(II) concentration (○) and cell protein content (□) in cultures; iron(II) concentration (●) and cell protein content (■) in cultures in the absence of ferric citrate. (**B**) The relationship between Fe(II) concentration and cell protein content.

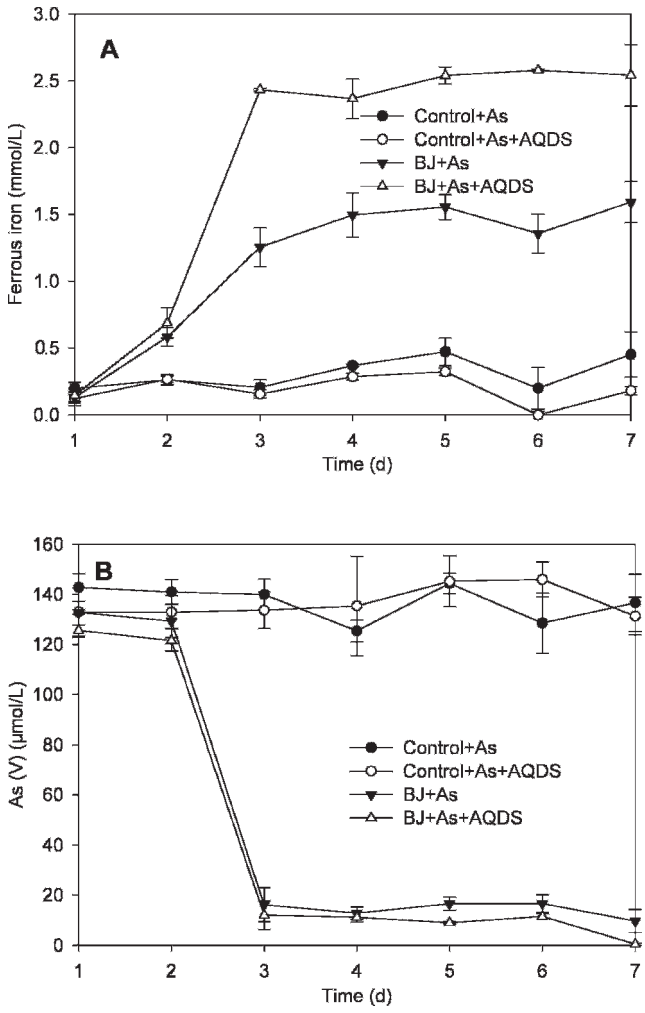

Fig. 4. (**A**) Growth of strain BJ in mineral medium with 3 mmol/L ferric citrate and 5 mmol/L acetate as the electron donor and supplemented with 150 μmol/L As(V) and 100 μmol/L anthraquinone-2,6-disulfonic acid (AQDS). The isolated *Aermononas* strain was designated as strain BJ. Data are means of triplicates; error bars represent standard error. (**B**) Effect of Fe(III) reduction on As immobilization in cultures.

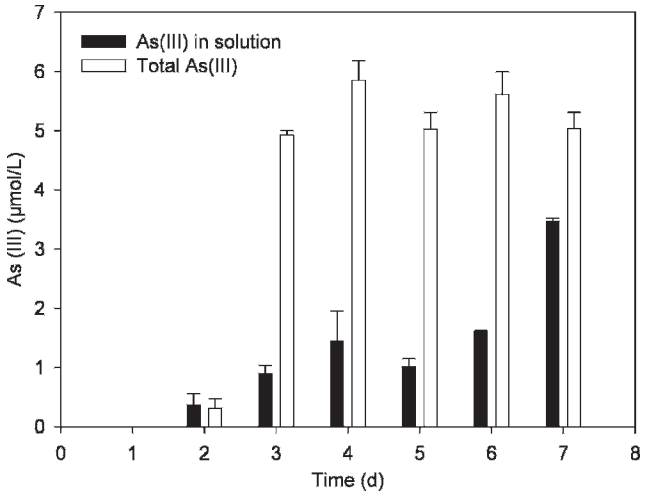

Fig. 5. Dissolved and total As(III) present during ferric citrate reduction.

the mineral was dominated by Fe minerals and high As contents. The As:Fe ratio in the precipitate was 1:13 (atom %) calculated based on energy-dispersive spectroscopy results (Fig. 7). X-ray diffraction analysis of the precipitate (Fig. 8) identified siderite and vivianite as the predominant minerals.

DISCUSSION

Plant root exudates and plant debris can release organic acids (e.g., citrate) that are known to chelate Fe(III). In comparison with dissolved Fe(III), iron plaque is composed primarily of ferrihydrite [29]. In addition, Liu et al. [18] demonstrated that As(V) predominated over As(III) in iron plaque. Therefore, in the present study, ferric citrate and ferrihydrite were used as iron sources for iron-reducing bacteria in the presence of As(V) to investigate the effect of microbial iron reduction on As mobility.

Arsenic mobilization by microbial reduction of Fe(III) minerals was implicated in previous studies [19,20,30]. However, in the experiments, alongside the production of Fe(II), a precipitate was formed in the culture containing ferric citrate, and As was removed from aqueous phase (Fig. 4B). During ferrihydrite reduction by strain BJ and in abiotic setups with ferrihydrite, As concentrations decreased sharply (Fig. 6). There was no significant difference ($p > 0.05$) between abiotic and biotic treatments. These results suggest first that aqueous As strongly binds to ferrihydrite, second that As can be removed by secondary Fe(II) phases, and third that reduction of As(V)-bearing Fe(III) oxides alone is not sufficient to mobilize As [31]. Although recent studies suggested that reductive processes indeed lead to As release to the aqueous phase over long periods of time [32], it is possible to control the reaction time and treatment systems as a means to maintain effective As retention.

In the present study, As(III) was detected in growing cultures of strain BJ containing 30 mmol/L Fe(III) citrate as the electron acceptor and supplemented with 150 µmol/L As(V). However, direct As(V) reduction by strain BJ was not found in an additional experiment that was conducted to determine whether strain BJ was able to couple anaerobic growth to the reduction of As(V) (data not shown). Thus, these results suggest that As(V) may be reduced abiotically by Fe(II) formed by strain BJ [33] or through As detoxification mechanisms in this organism [34]. The total As(III) in the

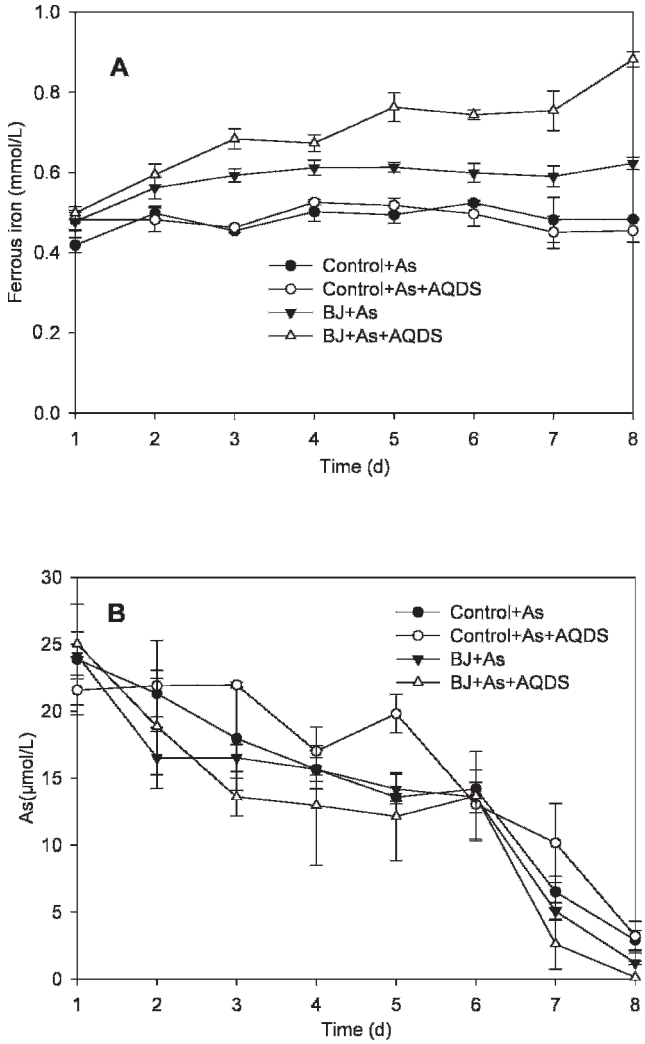

Fig. 6. (**A**) Growth of strain BJ in mineral medium with 7.5 mmol/L ferrihydrite and 5 mmol/L acetate as the electron donor and supplemented with 150 µmol/L As(V) and 100 µmol/L anthraquinone-2,6-disulfonic acid (AQDS). The isolated *Aermononas* strain was designated as strain BJ. Data are means of triplicates; error bars represent standard error. (**B**) Effect of Fe(III) reduction on As immobilization in cultures.

system did not significantly change; however, As(III) concentrations in solution showed a slightly increasing trend, indicating that As(V) reduction to As(III) favors As desorption from iron (hydr)oxides [35].

Some studies have shown that AQDS, a functional analog for quinone moieties in humic substances, can transfer electrons between microorganisms and iron minerals [36,37]. Thus, it can enhance the reduction of Fe(III) and the reductive dissolution of iron minerals [38]. In the present study, Fe(II) was produced at higher rates and to a larger extent in the presence of AQDS rather than in its absence, potentially leading to more stable minerals or even more crystalline iron minerals. Bacterial reduction of ferrihydrite to more crystalline minerals likely would lead to a significant decrease in the mineral surface area, potentially increasing As desorption [39]. Humic substances are widespread in upland and paddy soils [40], and iron oxyhydroxides are ubiquitously reactive constituents in subsurface environments, which are the preferred substrates for iron-reducing bacteria. Therefore, iron reduction via humic substances could be a possible way to

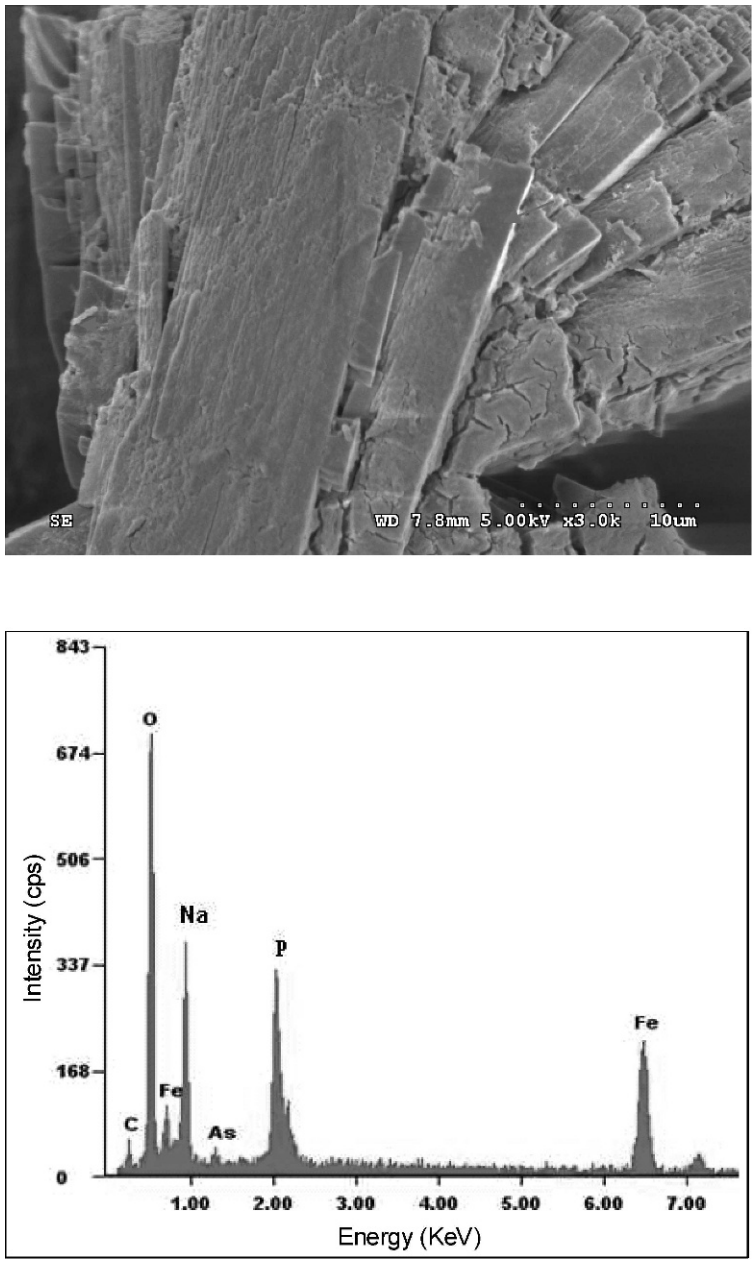

Fig. 7. Scanning electron micrograph and energy-dispersive spectroscopy analysis of mineral precipitates formed during reduction of ferric citrate by strain BJ. The arrows indicate the cells.

remove aqueous As from contaminated soils and thus reduce the potential risk for humans.

Aeromonas have been regarded as opportunistic pathogens of both animals and human, and their role in public health is well documented [41,42]. However, little attention has been paid to the physiology and ecology of this group. The present study suggests that strain BJ belonging to the *Aeromonas* genus can carry out anaerobic respiration with Fe(III). In a study by Knight and Blakemore [43], cell growth coupled to Fe(III) reduction by *A. hydrophila* was demonstrated, and no growth was observed in the absence of Fe(III). In their study, *A. hydrophila* showed poor Fe(III) reduction in the presence of amorphous Fe(III) oxyhydroxides. Neither anaerobic respiration nor Fe(III) reduction had been reported for members of the *Aeromonas* group isolated from paddy soil thus far. Other Fe(III)-reducing *Aeromonas* bacteria have been isolated from river sediments and heavy-metal-contaminated sites [43,44]. Therefore, the present study suggests that *Aeromonas* species also may be an important group of Fe(III)-reducing microorganisms along with *Geobacter* and *Shewanella* species.

Taking into account that strain BJ has been isolated from paddy soil, its predominant metabolism has yet to be investigated. Similarly, it remains to be determined whether this isolated iron reducer represents a member of the predominant populations of iron reducers in rice paddy soil. In addition, the role of strain BJ as an iron reducer in rice paddy soil has to be further studied in the future.

CONCLUSIONS

A member of the *Aeromonas* genus was isolated from paddy soils. Results of the present study showed that the isolated bacterium can carry out anaerobic respiration with dissolved and solid-phase Fe(III) in the presence of As(V), leading to the formation of Fe(II)-bearing minerals that strongly sorb As(V).

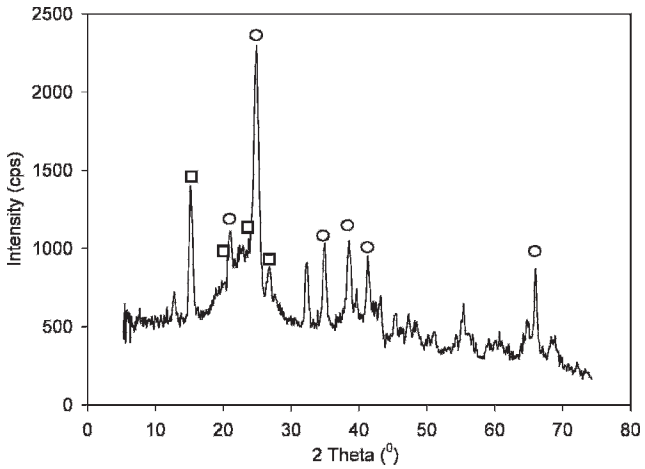

Fig. 8. X-ray diffraction analysis of the mineral precipitate formed by reduction of Fe(III) citrate by the isolated *Aermononas* strain. Symbols indicate vivianite (○) and siderite (□). cps = counts per second.

When the cells were growing with ferrihydrite in the presence of As(V), dissolved As concentrations decreased sharply. Moreover, our results indicated that AQDS could accelerate iron reduction by the isolated Fe(III)-reducing bacterium in the presence of As(V).

Acknowledgement—We are grateful to Katja Amstaetter and Christoph Berthold (University of Tübingen) for their assistance with X-ray diffraction analyses. The present study received financial support from the Chinese Academy of Sciences (KZCX1-YW-06-03).

REFERENCES

1. Lovley DR, Phillips EJP, Lonergan DJ. 1991. Enzymatic versus nonenzymatic mechanisms for Fe(III) reduction in aquatic sediments. *Environ Sci Technol* 25:1062–1067.
2. Lovley DR, Phillips EJP. 1986. Organic matter mineralization with reduction of ferric iron in anaerobic sediments. *Appl Environ Microbiol* 51:683–689.
3. Benner SG, Hansel CM, Wielinga BW, Barber TM, Fendorf S. 2002. Reductive dissolution and biomineralization of iron hydroxide under dynamic flow conditions. *Environ Sci Technol* 36:1705–1711.
4. Kappler A, Straub KL. 2005. Geomicrobiological cycling of iron. *Reviews in Mineralogy and Geochemistry* 59:85–108.
5. Peretyazhko T, Sposito G. 2005. Iron(III) reduction and phosphorous solubilization in humid tropical forest soils. *Geochim Cosmochim Acta* 69:3643–3652.
6. Lovley DR, Phillips EJP. 1988. Novel mode of microbial energy metabolism: Organic carbon oxidation coupled to dissimilatory reduction of iron or manganese. *Appl Environ Microbiol* 54:1472–1480.
7. Roden EE, Lovley DR. 1993. Dissimilatory Fe(III) reduction by the marine microorganism *Desulfuromonas acetoxidans*. *Appl Environ Microbiol* 59:734–742.
8. Weiss JV, Emerson D, Megonigal JP. 2004. Geochemical control of microbial Fe(III) reduction potential in wetlands: Comparison of the rhizosphere to non-rhizosphere soil. *FEMS Microbiol Ecol* 48:89–100.
9. Coates JD, Bhupathiraju VK, Achenbach LA, McInerney MJ, Lovley DR. 2001. *Geobacter hydrogenophilus*, *Geobacter chapellei* and *Geobacter grbiciae*, three new, strictly anaerobic, dissimilatory Fe(III)-reducers. *Int J Syst Evol Microbiol* 51:581–588.
10. Lin B, Braster M, Roling WFM, Van Breukelen BM. 2007. Iron-reducing microoganisms in a landfill leachate-polluted aquifer: Complementing culture-independent information with enrichments and isolations. *Geomicrobiol J* 24:283–294.
11. Weiss JV, Emerson D, Backer SM, Megonigal JP. 2003. Enumeration of Fe(II)-oxidizing and Fe(III)-reducing bacteria in the root zone of wetland plants: Implications for a rhizosphere iron cycle. *Biogeochemistry* 64:77–96.
12. Ratering S, Schnell S. 2000. Localization of iron-reducing activity in paddy soil by profile studies. *Biogeochemistry* 48:341–365.
13. Nickson RT, McArthur JM, Burgess WG, Ahmed KM, Ravenscroft P, Rahman M. 1998. Arsenic poisoning of Bangladesh groundwater. *Nature* 395:338.
14. Meharg AA, Rahman M. 2003. Arsenic contamination of Bangladesh paddy field soils: Implications for rice contribution to arsenic consumption. *Environ Sci Technol* 37:229–234.
15. Williams PN, Islam MR, Adomako EE, Raab A, Hossain SA, Zhu YG, Feldmann J, Meharg AA. 2006. Increase in rice grain arsenic for regions of Bangladesh irrigating paddies with elevated arsenic in groundwaters. *Environ Sci Technol* 40:4903–4908.
16. Chen Z, Zhu YG, Liu WJ, Meharg AA. 2005. Direct evidence showing the effect of root surface iron plaque on arsenite and arsenate uptake into rice (*Oryza sativa* L.) roots. *New Phytol* 165: 91–97.
17. Liu WJ, Zhu YG, Smith FA, Smith SE. 2004. Do iron plaque and genotypes affect arsenate uptake and translocation by rice seedlings (*Oryza sativa* L.) grown in solution culture? *J Exp Bot* 55:1707–1713.
18. Liu WJ, Zhu YG, Hu Y, Williams PN, Gualt AG, Meharg AA, Charnock JM, Smith FA. 2006. Arsenic sequestration in iron plaque, its accumulation and speciation in mature rice plants (*Oryza sativa* L.). *Environ Sci Technol* 40:5730–5736.
19. Horneman A, van Geen A, Kent DV, Mathe PE, Zheng Y, Dhar RK, O'Connell S, Hoque MA, Aziz Z, Shamsudduha M, Seddique AA, Ahmed KM. 2004. Decoupling of As and Fe release to Bangladesh groundwater under reducing conditions. Part I: Evidence from sediment profiles. *Geochim Cosmochim Acta* 68: 3459–3473.
20. Islam FS, Gault AG, Boothman C, Polya DA, Charnock JM, Chatterjee D, Lloyd JR. 2004. Role of metal-reducing bacteria in arsenic release from Bengal delta sediments. *Nature* 430:68–71.
21. Takahashi Y, Minamikawa R, Hattori KH, Kurishima K, Kihou N, Yuita K. 2004. Arsenic behavior in paddy fields during the cycle of flooded and non-flooded periods. *Environ Sci Technol* 38: 1038–1044.
22. Widdel F, Bak F. 1992. Gram-negative mesophilic sulfate reducing bacteria. In Balows A, Trüper HG, Dworkin M, Harder W, Schleifer KH, eds, *The Prokaryotes*. Springer, New York, NY, USA, pp 3352–3378.
23. Marmur J. 1961. A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* 3:208–218.
24. Straub KL, Kappler A, Schink B. 2005. Enrichment and isolation of ferric-iron- and humic-acid-reducing bacteria. *Methods Enzymol* 397:58–77.
25. Ying GH. 1993. Electron Microscope Technique and Cell Ultrastructure in Medicine and Biology. Hongkong Modern Press, Hongkong, China (in Chinese).
26. Ehrenreich A, Widdel F. 1994. Anaerobic oxidation of ferrous iron by purple bacteria, a new type of phototrophic metabolism. *Appl Environ Microbiol* 60:4517–4526.
27. Lu RK. 1999. *Analytical Methods for Soil and Agricultural Chemistry*. China Agricultural Science and Technology Press, Beijing, China (in Chinese).
28. Zhu YG, Sun GX, Lei M, Teng M, Liu YX, Chen NC, Wang LH, Carey AM, Deacon C, Raab A, Meharg AA, Williams PN. 2008. High percentage inorganic arsenic content of mining impacted and nonimpacted Chinese rice. *Environ Sci Technol* 42:5008–5013.
29. Hansel CM, Fendorf S, Sutton S, Newville M. 2001. Characterization of Fe plaque and associated metals on the roots of mine-waste impacted aquatic plants. *Environ Sci Technol* 35:3863–3868.
30. Cummings DE, Caccavo F, Fendorf S, Rosenzweig RF. 1999. Arsenic mobilization by the dissimilatory Fe(III)-reducing bacterium *Shewanella* alga BrY. *Environ Sci Technol* 33:723–729.
31. Islam FS, Pederick RL, Gault AG, Adams LK, Polya DA, Charnock JM, Lloyd JR. 2005. Interactions between the Fe(III)-reducing bacterium *Geobacter sulfurreducens* and arsenate, and capture of the metalloid by biogenic Fe(II). *Appl Environ Microbiol* 71:8642–8648.
32. Tufano KJ, Fendorf S. 2008. Confounding impacts of iron reduction on arsenic retention. *Environ Sci Technol* 42:4777–4783.
33. Bose P, Sharma A. 2002. Role of iron in controlling speciation and mobilization of arsenic in subsurface environment. *Water Res* 36: 4916–4926.
34. Seshadri R, Joseph SW, Chopra AK, Sha J, Shaw J, Graf J, Haft D, Wu M, Ren QH, Rosovitz MJ, Madupu R, Tallon L, Kim M,

Jin SH, Vuong H, Stine OC, Ali A, Horneman AJ, Heidelberg JF. 2006. Genome sequence of *Aeromonas hydrophila* ATCC 7966^T: Jack of all trades. *J Bacteriol* 188:8272–8282.
35. Tufano KJ, Reyes C, Saltikov CW, Fendorf S. 2008. Reductive process controlling arsenic retention: revealing the relative importance of iron and arsenic reduction. *Environ Sci Technol* 42:8283–8289.
36. Lovley DR, Coates JD, Blunt-Harris EL, Phillips EJP, Woodward JC. 1996. Humic substances as electron acceptors for microbial respiration. *Nature* 382:445–448.
37. Kappler A, Benz M, Schink B, Brune A. 2004. Electron shuttling via humic acids in microbial iron(III) reduction in a freshwater sediment. *FEMS Microbiol Ecol* 47:85–92.
38. Fredrickson JK, Kostandarithes HM, Li SW, Plymale AE, Daly MJ. 2000. Reduction of Fe(III), Cr(VI), U(VI), and Tc(VII) by *Deinococcus radiodurans* R1. *Appl Environ Microbiol* 66:2006–2011.
39. Tadanier CJ, Schreiber ME, Roller JW. 2005. Arsenic mobilization through microbially mediated deflocculation of ferrihydrite. *Environ Sci Technol* 39:3061–3068.
40. Dou S, Zhang JJ, Li K. 2008. Effect of organic matter applications on ^{13}C-NMR spectra of humic acids of soil. *Eur J Soil Sci* 59:532–539.
41. Hazen TC, Fliermans CB, Hirsch RP, Esch GW. 1978. Prevalence and distribution of *Aeromonas hydrophila* in the United States. *Appl Environ Microbiol* 36:731–738.
42. Rippey SR, Cabelli VJ. 1989. Use of the thermotolerent *Aeromonas* group for the trophic state classification of freshwaters. *Water Res* 23:1107–1114.
43. Knight V, Blakemore R. 1998. Reduction of diverse electron acceptors by *Aeromonas hydrophila*. *Arch Microbiol* 169:239–248.
44. Scala DJ, Hacherl EL, Cowan R, Young LY, Kosson DS. 2006. Characterization of Fe(III)-reducing enrichment cultures and isolation of Fe(III)-reducing bacteria from the Savannah River site, South Carolina. *Res Microbiol* 157:772–783.